Table 1. Percentage similarity and divergence of eight cytochrome P450s from various insects[a]

	CYP6A1[b]	*CYP6A2*[b]	*CYP6B1*[b]	*CYP6B2*[b]	*CYP6C1*[b]	*CYP6C2*[b]	*CYP6D1*[b]	*CYP6E1*[b]
CYP6A1	—	46.7	28.1	28.8	33.3	38.9	25.4	38.9
CYP6A2	78.8	—	31.9	31.7	34.5	38.5	27.4	35.7
CYP6B1	136.4	129.0	—	53.0	27.6	27.7	27.7	29.5
CYP6B2	136.8	126.3	68.3	—	27.4	27.4	25.4	28.7
CYP6C1	120.9	119.9	152.4	149.1	—	65.7	27.6	34.1
CYP6C2	104.6	107.7	145.3	146.1	44.2	—	27.7	38.3
CYP6D1	151.3	147.7	147.5	154.5	144.9	135.2	—	31.3
CYP6E1	100.1	111.5	140.5	143.8	116.7	100.7	128.7	—

[a] Figures in upper right and lower left portions of the table indicate percentage similarity and percentage divergence of amino acid sequences, respectively which were calculated using DNA star software (Madison, WI) via Clustal method of alignment.
[b] CYP6A1,[8] CYP6C,[9] CYP6C2[9] and CYP6D1[3] are derived from *Musca domestica*, CYP6A2[5] from *Drosophila melanogaster*, CYP6B1[10] is from *Papilio polyxes* and CYP6B2[4] from *Helicoverpa armigera*.

library and identified another cytochrome P450 cDNA from *C quinquefasciatus* larvae.

The PCR products of *c*250 bp were amplified, and 35 and 29 clones from permethrin-suseceptible and -resistant JPal-per strains, respectively, were sequenced. Alignments of the deduced amino acid sequences from these clones revealed that there were seven distinct sequences. One of these clones, which accounted for more than 57% of the total clones, was used as a probe to re-screen the cDNA library. Another full-length cDNA was cloned and designated CYP6F1. Northern blot analysis revealed that the *CYP6F1* gene in JPal-per strain appeared to be expressed more strongly than that in the S strain suggesting a possible involvement of this gene in resistance to pyrethroids. This result supports our previous finding that the content of cytochrome P450 was about 2.7 times higher in JPal-per strain than in the S strain.[1] Results of Southern blotting indicated that *CYP6F1* genes from susceptible and JPal-per strains contain different *Eco* RI and *Eco* RV digestion sites, suggesting that these two strains have different genome structures.

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Control of clubroot of crucifers by *Phoma glomerata* and its product epoxydon

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Abstract: *Phoma glomerata* strain JCM9972 con-

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trols clubroot of cruciferous crops caused by *Plasmodiophora brassicae* and the activity depends upon epoxydon (5-hydroxy-3-(hydroxymethyl)-7-oxabicyclo [4.1.0]hept-3-en-2-one) produced by the strain.

Keywords: clubroot; disease control; epoxydon; *Phoma glomerata*

A fungal strain isolated from *Viola* sp leaf sampled in Saitama, Japan was found to control clubroot disease of brassica crops caused by *Plasmodiophora brassicae* Woron. This pathogen is of world-wide significance, causing substantial damage to these crops, yet farmers and growers have few good control strategies. Pentachloronitrobenzene (PCNB) has been used frequently as an effective fungicide to control the disease. However, PCNB and its major metabolite pentachloronitroaniline are stable and persist in the soil for a long period after application, so that development of some alternative control method is desirable.[1]

Clubroot control by the isolated fungal strain could be obtained either by mixing the mycelia cultured on soil–bran medium (10% of the infested soil) or by pouring the culture broth (in potato dextrose medium; 30 ml per 200 g soil) into the infested soil.[2] Development of galls on the root of Chinese cabbage (*Brassica rapa pekinensis* group), turnip (*B rapa rapifera* group), cabbage (*B oleracea capitata* group), and broccoli (*B oleracea italika* group) was suppressed by both treatments. The effect occurred with all the soil types tested, ie horticultural, alluvium, and diluvium soil. The fungal strain forms a pale to dark-brown colony on potato dextrose agar medium with non-septate and hyaline conidia in pycnidia which are globose to subglobose and with a beak. It also forms *Alternaria*-like, brown to dark-brown, variable in shape and size, catenated, and often branched dictyochlamydospores on mycelia. With these morphological characters the strain was identified as *Phoma glomerata* (Corda) Wollenw & Hochapfel,[3] and it has been deposited as JCM9972 in the Japan Collection of Micro-organisms, RIKEN.

P glomerata JCM 9972 culture broth in potato-dextrose medium (23 litres) yielded 1.02 g of the pure active substance after the series of chromatographic procedures depicted in Fig 1. Spectroscopy (HR-MS, EI-MS, [^{1}H]NMR and [^{13}C]NMR) allowed identification of the active compound as epoxydon, 5-hydroxy-3-(hydroxymethyl)-7-oxabicyclo [4.1.0] hept-3-en-2-one (Fig 2).[2,4] This compound was initially isolated from *Phoma* sp culture broth by Closs, Mauli and Sigg in 1966 as an anti-tumour agent.[4] They reported an EC_{50} of 1 μg ml^{-1} against mastzelltumors in mice with acute toxicity to mice (LD_{50}) of 70 mg kg^{-1}.

In our experiments, epoxydon showed neither strong anti-microbial activity at 100 μg ml^{-1} against many fungi and bacteria, such as *Fusarium oxysporum*

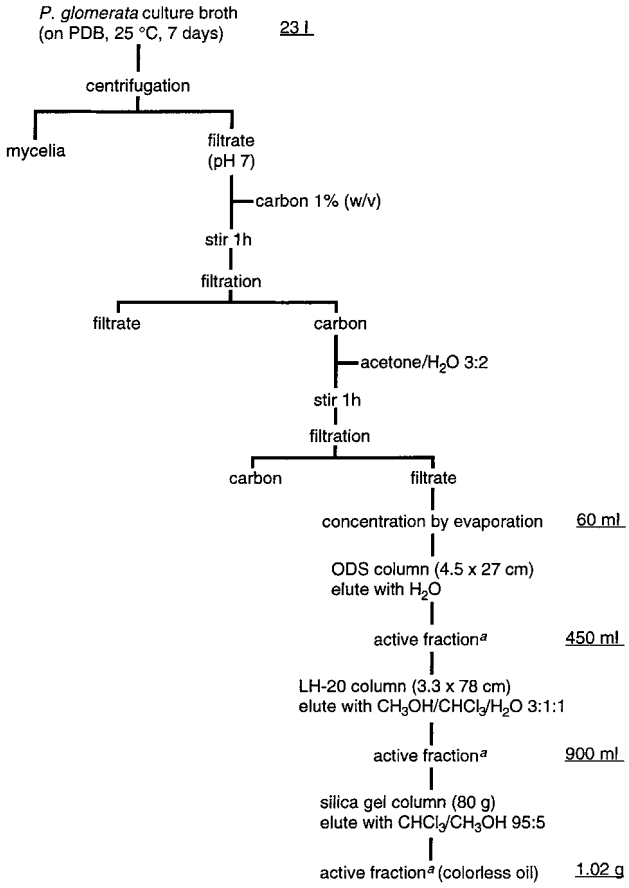

Figure 1. Pictorial explanation for purification procedure of active substance from *Phoma glomerata* JCM9972 culture broth. [a]Assessed by clubroot reduction.

Schlecht, *Alternaria mali* Roberts, *Botrytis cinerea* Pers, *Burkholderia caryophylli*, and *Xanthomonas campestris* Dye, nor induction of acquired resistance in the plant, yet it could prevent clubroot of crucifers at 100–250 μg ml^{-1} by pouring the culture broth into the infested soil (30 ml per 200 g soil). As epoxydon has been reported to have an anti-auxin activity,[5] several known anti-auxins were tested for the suppression of clubroot disease, and some of them, eg, 2,3,5-triiodobenzoic acid, showed significant control efficacy against clubroot. Since *P brassicae*-

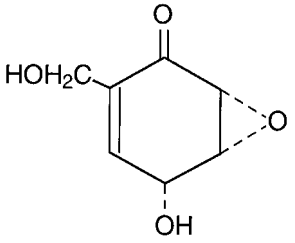

Figure 2. Chemical structure of epoxydon.

infected plant galls contain excessive amounts of auxin in comparison with the healthy roots,[6–8] auxin may play an important role in gall formation, and anti-auxin substances might well suppress the disease. This suggests that the clubroot suppression activity of epoxydon is due to the anti-auxin activity.

Recently, *Phoma* spp have been recognized as fungi with plant growth promoting activity (PGPF).[9] The *P glometara* JCM9972 strain seems to be one of them and its application as a biocontrol agent is worth investigation.

P glomerata JCM9972 and epoxydon may constitute a new group of plant protection agents with no fungicidal activity. Hence, this study, while focusing on the control of clubroot, may be much more widely applicable. It is likely that these chemicals could be environmentally compatible and might not produce resistant strains of pathogens.

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Ion regulation in the larval lepidopteran midgut and the response to *Bacillus thuringiensis* δ-endotoxin

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Abstract: Fourth-instar *Bombyx mori* (silkworm) was used as a model insect to study the effects of *Bacillus thuringiensis* (Bt) on ion homeostasis in the larval lepidopteran midgut. The K^+ chemical gradient across the midgut of *B. mori* larvae is quite small, sustained by a lumen/haemolymph activity ratio of only 1.6. More than 95% of the driving force causing K^+ flux from lumen to haemolymph is electrical. In contrast to K^+, the H^+ chemical gradient is exceedingly large, with luminal pH values of 11–12 and haemolymph/lumen H^+ ratios as high as 10^5. At equilibrium, the steep proton gradient is consistent with a passive distribution of H^+ across the midgut epithelium as predicted from the Nernst equation. In *B mori* larvae, ingestion of a lethal dose of Bt δ-endotoxin produces an increase in K^+ conductance in the midgut apical epithelium, causing a decrease in the electrical gradient and dissipation of the pH gradient. Larval morbidity can be correlated with a rise in haemolymph K^+ and pH and a decline in luminal pH. Midgut K^+ activity, however, remains unchanged. An important factor in the pathogenesis of Bt is irreversible alkalization of the epithelial cells as H^+ is redistributed across the midgut to reach a new Nernst equilibrium.

Keywords: *Bacillus thuringiensis*; Lepidoptera; midgut; ion regulation; potassium; pH

1 INTRODUCTION

The pH of the larval lepidopteran midgut is one of the highest known for any biological system. Values in the range pH 10–11, and at times as high as pH 12, have been recorded in *Bombyx mori* L and other species.[1] This extreme alkalinity is thought to be an evolutionary adaptation to a tannin-rich leaf diet, and is mediated by secondary active transport of K^+ into the midgut lumen by goblet cells. The present model for this process is based on an ATP-dependent, electrogenic, primary H^+ pump coupled with an electrophoretic $1K^+/2H^+$ antiporter in the goblet cell apical membrane (GCAM).[2] The H^+ pump is a vacuolar-type ATPase visible as portasomes (10-nm particles) lining the cytoplasmic side of the GCAM.[3] The exchange of H^+ for K^+ by the antiporter results in K^+, rather than H^+, being pumped into the midgut lumen, allowing for alkalization, rather than acidification, of the luminal contents. Overall electroneutrality is maintained by secretion of carbonate into the lumen

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